Decision Making: Singin' in the Brain

Richard Benton[1,*]
[1]Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, CH-1015 Lausanne, Switzerland
*Correspondence: richard.benton@unil.ch
DOI 10.1016/j.neuron.2011.01.021

Which neurons in the brain "decide" to initiate particular behaviors in response to sensory information? In this issue of *Neuron*, two papers (Kohatsu et al. and von Philipsborn et al.) identify candidates in the courtship circuitry of *Drosophila*. The activity of these neurons is both regulated by sex pheromones and necessary and sufficient to trigger male love song.

You're offered alternative options ("Tea or coffee?"), assign and compare their value ("I prefer coffee …"), picture the consequences of making a choice based upon experience ("… but it is getting late …"), and then, all of a sudden, you've made a decision. What is the neural basis for how we decide? Psychological and neurophysiological studies in humans and nonhuman primates have provided fundamental understanding of the steps of the decision-making process and their associated brain regions (Kable and Glimcher, 2009), but higher-resolution analysis in these animals presents significant technical challenges. Organisms with much simpler nervous systems must also make choices, such as that of leeches to swim or crawl in shallow waters (Kristan, 2008), or those of nematode worms when evaluating potential food sources (Rankin, 2006). While these model systems may not exhibit the depth of our conscious reflections, they open the possibility to characterize the contributions of individual neurons to the decision-making process and, thereby, perspectives into ancestral cellular mechanisms of this important property of neural circuits.

The fruit fly, *Drosophila melanogaster*, is a particularly attractive experimental system to study decision-making because it offers powerful genetic tools to control (and monitor) the function of small populations of neurons in the brain and determine the effect on simple behavioral choices in intact animals (Olsen and Wilson, 2008). One of the most important decisions for *Drosophila* is—as in many other organisms—with whom to mate (Dickson, 2008; Manoli et al., 2006). A male may frequently encounter another fly when feeding upon a rotting fruit but only rarely decide to court it. He first determines the species, sex, and mating status of the target, primarily by sensing volatile and contact pheromones. These chemical signals can be either stimulatory (from females) or inhibitory (from other males) and are thought to activate hard-wired circuits to control the decision to court (Dickson, 2008). However, the male is also influenced—as in many other animals—by memories of his previous sexual experiences, particularly the unsuccessful ones (Griffith and Ejima, 2009). A male that decides to court then engages in an elaborate behavioral ritual to entice a female, most notably in the performance of a courtship song. Produced by the vibration of one wing, this serenade is composed of two motifs: sine song and pulse song. The latter is important for a female to determine whether her suitor is of the same species (Murthy, 2010).

Male courtship behavior—and the decision to initiate it—is controlled in large part by about 2000 neurons that express the sex-specific transcription factor Fruitless^{M} ("Fru^{M} neurons") (Dickson, 2008; Manoli et al., 2006). These neurons encompass sensory cells that detect pheromones, interneurons in higher brain centers, and motor neurons, including those in the ventral nerve cord (VNC) in the thorax that control song production (Cachero et al., 2010; Kimura et al., 2008; Yu et al., 2010). Inhibition of all Fru^{M} neurons prevents courtship in males, indicating their necessity for this behavior (Dickson, 2008; Manoli et al., 2006). Conversely, optogenetic activation of Fru^{M} neurons in the VNC in decapitated males is sufficient to induce singing, suggesting that these thoracic Fru^{M} neurons function as regulators or integral components of the central pattern generator for song (Clyne and Miesenböck, 2008). Surprisingly, beheaded females can also be induced to sing—albeit slightly out of tune—when equivalent VNC neurons are activated (Clyne and Miesenböck, 2008). Given the presence of a latent song generator in both sexes that is normally activated only in males exposed to female pheromones, which neurons in the brain make the decision to sing?

The groups of Barry Dickson (von Philipsborn et al., 2011) and Daisuke Yamamoto (Kohatsu et al., 2011) addressed this question by modifying the gain-of-neural-function approach established previously (Clyne and Miesenböck, 2008). Using complementary intersectional and clonal expression strategies, both teams expressed the heat-sensitive ion channel, TrpA1, in small, distinct subsets of Fru^{M} neurons in hundreds of different flies. They then screened these animals to identify those in which heat-induced depolarization of the TrpA1-expressing neurons was sufficient to induce males to sing in the absence of females. Satisfyingly, these screens converged in their identification of a cluster of ~20 interneurons, named P1, whose activation induced robust and apparently accurate pulse song production, as well as other elements of male courtship behavior, such as abdominal bending.

P1 neurons have two important properties (Figure 1): first, they are located in the lateral protocerebrum, a higher brain center that receives sensory input from olfactory, gustatory, visual, and auditory systems. Second, P1 neurons are present only in males. Thus, these cells appear to be ideal candidates to integrate multimodal environmental stimuli to make the

decision to court in males, but not in females. Earlier work from the Yamamoto laboratory had, in fact, already implicated P1 neurons in regulating male courtship (Kimura et al., 2008); in that study, they found that selective masculinization of the female lateral protocerebrum—by generating clones mutant for *transformer*, a regulator of sex determination—resulted in ectopic appearance of P1 neurons and a low level of male courtship-like behavior in these otherwise female individuals. On the other hand, conditional inhibition of synaptic transmission in P1 neurons in the male brain reduced singing and other courtship elements (Kimura et al., 2008), findings that are confirmed and extended in the new work (Kohatsu et al., 2011; von Philipsborn et al., 2011). Thus, activity of P1 neurons is both necessary and sufficient to trigger male love song production. Moreover, because they do not appear to influence the structure of pulse song and also play a role in initiating other courtship behaviors, these interneurons may form part of the decision center in the courtship circuitry.

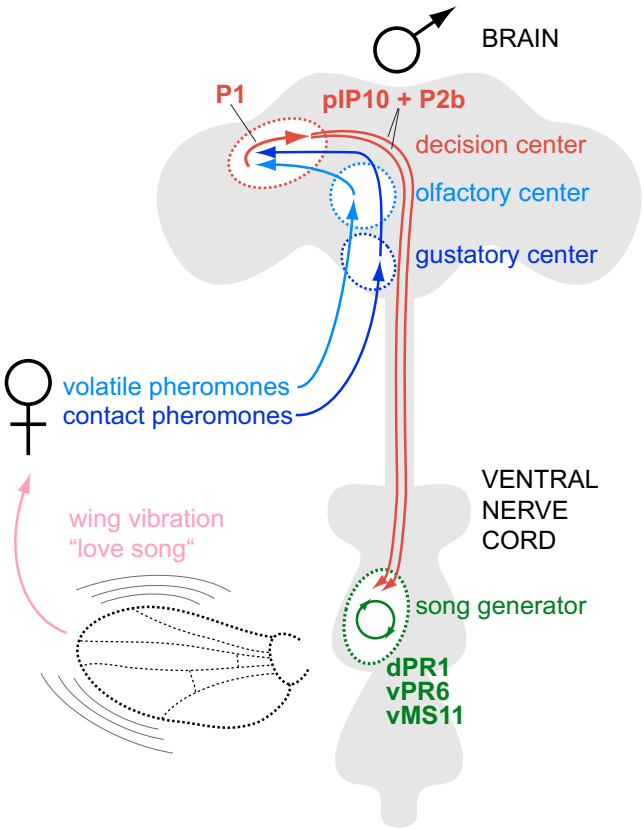

Figure 1. A Schematic Circuit Underlying Male Love Song Production

How do P1 neurons integrate functionally into a decision-making circuit? Kohatsu et al. (2011) looked upstream by asking whether their physiological activity is regulated by sensory stimuli that control male courtship. To do this, they developed a versatile "tethered male" preparation in which courtship behavior towards a specific object can be assessed simultaneously with optical imaging of neural activity in the brain. Presentation of a female, but not male, fly to the tethered animal was sufficient to trigger many characteristic elements of the courtship ritual, including wing vibration. Notably, initiation of robust behavioral responses required physical contact between the male and the female, suggesting that gustatory, rather than olfactory or visual, stimuli provide the cue to trigger this behavior. Indeed, extracts from female cuticles (which contain sex pheromones [Ferveur, 2005]) were also sufficient to evoke courtship initiation, although the behavioral response did not persist in the absence of other stimuli. Using the genetically encoded calcium sensor, Cameleon, these authors then showed that P1 neurons displayed rapid calcium increases upon contact of the male with a female, consistent with the hypothesis that P1 neurons mediate the decision to initiate courtship upon receipt of sensory signals from female pheromones.

Courtship is also regulated by the volatile chemical *cis*-vaccenyl acetate. Produced in the male ejaculatory bulb and transferred to females during copulation, this pheromone is detected by the olfactory system to inhibit male courtship of other males and mated females (Dickson, 2008; Griffith and Ejima, 2009). Kohatsu et al. (2011) showed that perfuming of females with *cis*-vaccenyl acetate (mimicking nonvirginity) reduces their ability both to physiologically stimulate P1 neurons and to provoke courtship behavior. This correlation is suggestive that the P1 cluster integrates olfactory and gustatory sensory cues when weighing up the decision to court or not (Figure 1).

von Philipsborn et al. (2011) focused their attention on circuit elements downstream of P1 neurons. Through their original thermogenetic screen, they identified four additional classes of Fru^M neurons whose activation was sufficient to trigger wing extension or vibration. One of these, named pIP10, was male-specific and both necessary and sufficient to reproduce a faithful rendition of male pulse song, similar to the properties of P1 neurons. However, unlike P1, pIP10 neurons innervate both higher brain centers (including the lateral protocerebrum) and the VNC, thus representing a putative descending (or "command") neuron that transmits signals from the brain to initiate song (Figure 1). Other types of descending neurons are likely to exist, for example, those that select sine versus pulse song, or control song termination. One of these may be P2b neurons, which Kohatsu et al. (2011) identified in their screen as being sufficient, although only partially necessary, to induce wing vibration.

The other three Fru^M neuron classes characterized by von Philipsborn et al. (2011), dPR1, vPR6, and vMS11, were distinct from P1 and pIP10 in two significant ways: first, activation of these neurons did not lead to faithful recapitulation of pulse song. For example, vMS11 activation induced wing extension but no singing, while vPR6 activation led to pulse song with a novel temporal structure. Second, all three types are contained within the VNC. These neural classes therefore represent candidate components or direct regulators of the song generator, and may correspond to some of the neurons previously shown to be

sufficient to induce singing in decapitated males and females (Clyne and Miesenböck, 2008). Indeed, while dPR1 is male-specific, vPR6 and vMS11 are present in both sexes, albeit exhibiting sexually dimorphic arborizations within the wing neuropil. Furthermore, the impact of vPR6 on pulse song patterning suggests these neurons are a "mutable" part of the song generator that might account for the diversity in courtship serenades critical for species recognition (Murthy, 2010).

While physiological evidence for functional connections between P1, pIP10, and the thoracic Fru^M neurons awaits, von Philipsborn et al. (2011) assess overlap between axonal and dendritic arbors of these neural classes to predict potential synaptic contacts. Their observations suggest—though do not prove—that these neurons are likely to form an interconnected circuit.

Together, these studies provide us with an excellent—though incomplete—neural framework to understand how converging sensory inputs are interpreted to induce a selection between alternative behavioral outputs. The available data point to the P1 cluster as the critical central neurons that trigger singing (and other aspects of the courtship routine), but how might these neurons weigh up positive and negative sensory influences on the decision to initiate courtship? A hint is offered by finer-scale thermal activation experiments of von Philipsborn et al. (2011), who found that at least ten out of 20 individual P1 neurons must express TrpA1 to induce singing. While it is unknown whether these cells are functionally homogeneous, it is intriguing to speculate that attainment of this threshold number of activated P1 neurons in wild-type flies may be what tips the balance in their mind in favor of courting. Future high-resolution anatomical mapping and physiological characterization of excitatory and inhibitory synaptic inputs to these neurons from different sensory systems, as well as their precise output pathways may reveal the cellular mechanisms by which neural circuits make decisions.

REFERENCES

Cachero, S., Ostrovsky, A.D., Yu, J.Y., Dickson, B.J., and Jefferis, G.S. (2010). Curr. Biol. *20*, 1589–1601.

Clyne, J.D., and Miesenböck, G. (2008). Cell *133*, 354–363.

Dickson, B.J. (2008). Science *322*, 904–909.

Ferveur, J.F. (2005). Behav. Genet. *35*, 279–295.

Griffith, L.C., and Ejima, A. (2009). Learn. Mem. *16*, 743–750.

Kable, J.W., and Glimcher, P.W. (2009). Neuron *63*, 733–745.

Kimura, K., Hachiya, T., Koganezawa, M., Tazawa, T., and Yamamoto, D. (2008). Neuron *59*, 759–769.

Kohatsu, S., Koganezawa, M., and Yamamoto, D. (2011). Neuron *69*, this issue, 498–508.

Kristan, W.B. (2008). Curr. Biol. *18*, R928–R932.

Manoli, D.S., Meissner, G.W., and Baker, B.S. (2006). Trends Neurosci. *29*, 444–451.

Murthy, M. (2010). Curr. Opin. Neurobiol. *20*, 281–287.

Olsen, S.R., and Wilson, R.I. (2008). Trends Neurosci. *31*, 512–520.

Rankin, C.H. (2006). Curr. Biol. *16*, R89–R91.

von Philipsborn, A.C., Liu, T., Yu, J.Y., Masser, C., Bidaye, S.S., and Dickson, B.J. (2011). Neuron *69*, this issue, 509–522.

Yu, J.Y., Kanai, M.I., Demir, E., Jefferis, G.S., and Dickson, B.J. (2010). Curr. Biol. *20*, 1602–1614.

Synaptotagmin 4: A New Antiobesity Target?

Qingchun Tong[1,*]
[1]Center for Diabetes and Obesity Research of the Brown Foundation Institute of Molecule Medicine and Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA
*Correspondence: qingchun.tong@uth.tmc.edu
DOI 10.1016/j.neuron.2011.01.018

In this issue of *Neuron*, Zhang et al. show that Synaptotagmin 4 (Syt4) is specifically induced in adult hypothalamic oxytocin neurons by high-fat diet. Evidence is provided to support a critical role for Syt4 in negative regulation of oxytocin release, which in turn is responsible for diet-induced obesity, raising the possibility of using Syt4 as a new antiobesity target.

Given the increasing prevalence of obesity and the devastating comorbidities associated with obesity, identifying effective antiobesity strategies is more imperative than ever. Although the underlying causes of the obesity epidemic are multifactorial, exposure to high-caloric diet (Western diet) is thought to be one of the major reasons. In order to mimic human obesity in animal models, a widely accepted strategy involves inducing obesity in rodent models with high-fat diet (HFD) feeding. The HFD feeding can induce obesity and metabolic disorders in rodents that resemble the human metabolic syndrome (Buettner et al., 2007). Thus, important antiobesity drug targets can be identified with HFD-induced obesity models.

Research efforts in the last decades have established that the hypothalamus